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EVALUATION OF EFFECTIVE RATE OF REARING (ERR), FILAMENT LENGTH AND DENIER OF NEW BREEDING LINES OF BOMBYX *MORI* L

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ABSTRACT

The present study was designed with the main purpose of developing bivoltine breeds for our tropical climatic conditions by using silkworm breeds with known genetic backgrounds (KA, NB_{18} and PM) in various hybrid combinations and incorporating them over generations, followed by backcrossing and adequate selection of different generations with the objective of profitability and productivity. The isolated Bivoltin lines (R_1 and R_2) were reared with their parental races at different times of the year to evaluate their stability in the expression of commercial traits. For the present breeding program, the purebred Bivoltine Kalimpong-A (KA), which spin white oval cocoons, New $Bivoltine_{18}$ (NB_{18}) white cocoons with rotating dumbbells and Multivoltine Pure Mysore (PM), the yellow pointed cocoons of the mulberry silkworm Bombyx mori L., Selected. One-way and three-way crosses were made using the above three breeds. The first single cross comprised KA females and PM males. The second unique cross comprised NB_{18} females and PM males. Selection was performed at the egg, larva, pupal, and cocoon stages over the course to determine the desired traits. The offspring of F from the respective crosses were backcrossed with their respective bivoltine males to improve commercial traits. Heterosis in the F_1 generations of crosses, including NB_{18} and PM, was determined by the mean score of the parents (MPV) and the best score of the parents (BPV). A significant test for heterosis was performed using a standard ANOVA table. Based on the results of metric traits viz. effective rate of rearing (ERR), filament length and denier it was delineated that Selection may be made to choose intermediate sized cocoons from the batches of high ERR because these individuals are expected to possess maximum fitness value.

KEYWORDS: Bombyx mori L, Bivoltine, ERR, Denier, Filament Length

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Original Article

INTRODUCTION

Among insects, Drosophila and domesticated mulberry silk worm Bombyx mori have been used extensively for various experimental purposes. The silk worm Bombyx mori, for a long time, has been under the patronage of man due to its economic value, and this has been an object of research since medieval times.[1] sericulture probably was practiced in China since 2255 BC and gradually spread to South Korea, Japan and by the North Eastern route to India in 140 BC via the so called 'Silk Road', a road ultimately leading to world culture.[2] Since the birth of silkworm genetics, extensive investigations have been carried out with to the objective of improving the economic characteristics. These results, together with silkworm breeding experiments, contributed much to the establishment of contemporary silkworm breeds of high economic values.

In India, sericulture is a domestic agricultural industry that mainly provides employment to the weaker sectors of society. Due to its employment potential and profitability, its low investment costs and its high foreign exchange earnings, silkworm farming could become an important factor for the economic development of our country. Therefore, studies of the genetic, reproductive and biochemical aspects of the silkworm will have a direct impact on the development of the silkworm industry.

There are many species of silkworm which produce cocoons of superior filament quality and containing larger quantities of silk. Among them Bombyx mori L. of the family Bombycidae is the only species widely used for commercial rearing. India holds unique position in the world by producing all the four commercially known varieties of silk namely, Mulberry, Eri, Tasar and Muga. India ranks second in raw silk (12,665 tonnes) production in the world (Statistical Biennial CSB 2017). The mulberry silk production in India accounts for 90.7% (28, 523 tonnes) while non-mulberry silks (eri 5060 tonnes 4.9%, Tasar 2819 tonnes) 3.8% and muga (166 tonnes 0.5%) account for 9.3% of the total raw silk production. The major share of the Indian mulberry silk production is from Karnataka accounting for 54.095% (6214 tonnes) (Statistical Biennial CSB 2017).

Systematic breeding experiments of silkworm evolved during the past few years have enabled the breeders to synthesise desirable genotypes of known genetic constitution with a main objective of increasing the productivity and viability. Application of conventional hybridization methods with appropriate selection have contributed a great deal in increasing quality and quantity of silk. Sericulturally advanced countries such as Japan and China have achieved a remarkable breakthrough in increasing the unit production of high grade silk by evolving silkworm races suitable to their agroclimatic conditions.[3] In India several attempts have been made during the last two decades to synthesise suitable, high productive bivoltine races and also multivoltine races to our tropical climatic conditions but with little success.[4-8] Further, in the absence of suitable bivoltine races, the locally available multivoltine and bivoltine races are being used for the production of commercial hybrids. Therefore, the unit production and the quality of silk produced by the commercial hybrids remains poor. Hence, it is essential to synthesise better bivoltine races for commercial exploitation under tropical climatic conditions. Moreover, voltinism, moultinism, viability, productivity, resistance or susceptible to disease and tolerance to unfavourable environmental conditions assume a special importance in the efforts to synthesise suitable silkworm races.

With this viewpoint, the present study was undertaken to evolve bivoltine races for our tropical climatic conditions by utilizing the silkworm races of known genetic background (KA, NB_{18} and PM) in different hybrid combinations and to inbreed them over generations followed by backcrossing and adopting appropriate selection at different generations with an objective of the viability and productivity. The isolated bivoltine lines (R_1 and R_2) were reared in different seasons of the year along with their parental races to evaluate their stability in the expression of commercial characters.

MATERIALS AND METHODS

Silkworm Varieties and Rearing

The pure races of bivoltine Kalimpong-A (KA) spinning oval white cocoons, New Bivoltine-18 (NB_{18}) spinning dumbbell white cocoons and multivoltine Pure Mysore (PM) spinning pointed yellow cocoons of mulberry silkworm Bombyx *mori* L. were selected for the present breeding programme. These races were obtained from their respective seed areas and are reared in cytogenetics laboratory, Jnana Bharathi, Bangalore University.

The disease free layings were prepared as described by Narasimhanna, (1988),[9] and were incubated at 25°C and relative humidity of 60-70%. On 8th day composite layings were prepared (10-20 layings were prepared 100-200 eggs were collected from each laying). The hatched worms were reared according to the method described by Krishnaswamy (1978).[10] MS variety of mulberry leaves were used in rearing. The worms were reared in mass up to III instar, after III moult 300 worms were collected in three replicates in order to evaluate the rearing performance. Standard temperature and humidity were maintained in the rearing house. The quantitative traits such as fecundity and hatching percentage were evaluated to assess the performance breeding lines of Bombyx *mori* L.

Breeding

Single and three way crosses were made by using the above said three races. The first single cross involved KA females and PM males. The second single cross involved NB_{18} females and PM males. During the course of breeding selection was made at the egg, larva, pupa and cocoon stages to fix the desirable traits. F_5 progenies of the respective crosses were back crossed to their respective bivoltine males to improve commercial characters.

Evolutions of New Lines R_1 and R_2

Females of KA and NB_{18} were crossed with males of PM. The composite layings of F_1 hybrid were brushed and reared under standard laboratory conditions. The selection parameters explained earlier were applied to choose the seed cocoons for the preparation of F_2 layings. The replicates showing higher pupation rate were selected for intra family selection of cocoons. Further, segregation with respect to cocoon colour and built was noticed. Only white oval in case of KAxPM and dumbbell white in case of NB_{18}xPM qualifying the parameter of selection were chosen for breeding in subsequent generations. The females of F_5 were backcrossed to the males of KA and NB_{18} respectively in both the lines and reared up to 11 generations. At the end of the 11th generation the lines R_1 and R_2 were extracted with higher ERR than their respective better parents, with shorter larval period and with moderate cocoon productivity character in case of R_1 and R_2.

Table 1

Breeding Plans I and II													
				I							II		
	KA	O	O	x	PM	Cto		NB18	O	O	x	PM	Cfo
		+	i-						+	+			
				F1							F1		
				F2							F2		
				F3							F3		
				F4							F4		
F5	x	KA	O	➘	er'		F5x	NB18	Cta	+			
				F1							F1		
				F2							F2		
				F3							F3		
				F4							F4		
				F5							F5		
				F6	(R1)						F6	(R2)	

Statistical Analysis

Heterosis in F_1 generations of crosses including NB_{18} and PM were determined over Mid-parental value (MPV) and better parent value (BPV).Heterosis was determined as follows;

% Heterosis over MP = (F1 – MPV/MPV) X 100

% Heterosis over BP = (F1 – BPV/BPV) X 100

Where;

MP: Mid Parent; BP: Better Parent

Significant test for heterosis was performed using standard ANOVA table. To test the generation effect on the rearing performance standard regressions of different parameters on the generation number were worked out. To compare the generation performance of crosses, the data were transformed into standard normal varieties. This is due to the fact that per second comparison of the absolute value does not show the inherent trend in the data.

RESULTS AND DISCUSSION

Effective Rate of Rearing (ERR)

The F_1 hybrids of the cross KAxPM revealed the mean ERR of 86±2.9% which increased to 89% in F_2, later decreased in succeeding generations. The F_5 females with mean ERR of 85.6±2.4% were back crossed to the KA males with an ERR of 73.6±4.9%. In F_l generations the ERR was 88 1.41%, it decreased in succeeding generations and again increased in F_6 generations (Table 2 and 3). Further it can be seen from Fig. 1 that the curve shows leading to the fixation of the character.

Table 2: Mean±S.D of Important Characters of Silkworm Hybrid KAxPM in 5 Generations

Generations	ERR (%)	FILL (ft.)	DEN
F1	86.000±2.944	883.667±35.780	2.200±0.00E+00
F2	89.000±2.944	828.333±38.801	2.267±4.71E-02
F3	84.667±1. 247	791.333±31.095	2.200±0.00E-00
F4	86.667±2.867	811.000±20.216	2.200±0.00E-00
F5	85.667±2.494	792.333±34.702	2.133±4.71E-02

Table 3: Mean±S.D of Important Characters of Silkworm Hybrid F (KAxPM) x KA in 6 Generations

Generations	ERR (%)	FILL (ft.)	DEN
F1	88.000±1.414	882.667±35.462	2.333±4.61E-02
F2	87.667±2.494	946.333±56.388	2.367±4.71E-02
F3	88.000±3.559	936.333±37.142	2.300±0.00E-00
F4	78.000±2.160	879.000±32.073	2.233±4.71E-02
F5	83.667±2.625	910.000±28.577	2.300±0.00E+00
F6	85.000±1.633	886.000±36.615	2.300±0.00E+00

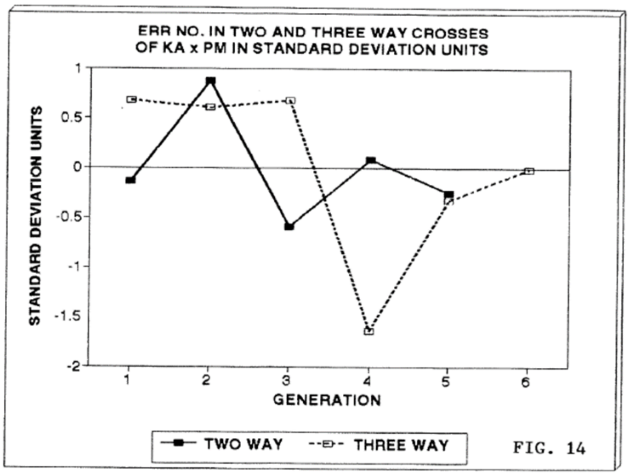

Figure 1

The F_1 hybrids of the cross NB_{18}xPM revealed a mean ERR of 81±1.73 which increased in succeeding generations. The F_5 females with a mass of ERR of 88% were back crossed to the NB_{18} males. In F_1 the ERR was found to be 89.3±2%. It increased in F_2 generation and again increased and it remained constant in F_5 and F_6 generations showing the fixation of the trait leading to the isolation of the line R_2. From Fig. 2 the curve shows increasing trend after F_3 generations (Tables 4 and 5).

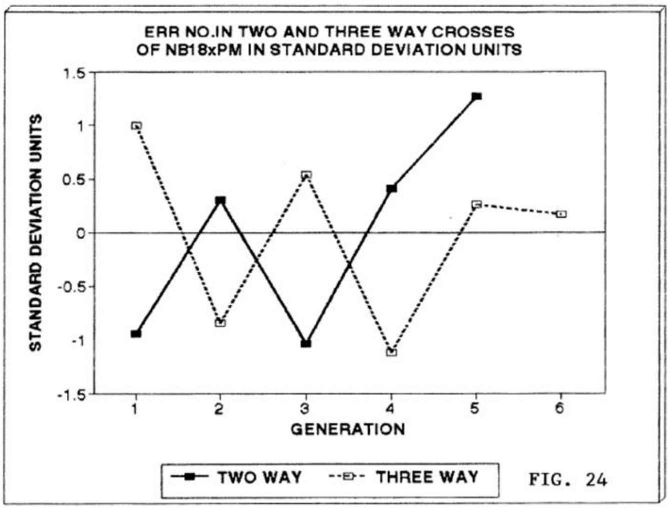

Figure 2

Table 4: Mean±S.D of Important Characters of Silkworm Hybrid NB1axPM in 5 Generations

Generations	ERR (%)	FILL (ft.)	DEN
1	81.000±1.732	876.667±41.633	2.167±0.057E-06
2	85.333±1.155	832.000±27.785	2.100±5.2E-08
3	80.667±2.082	834.000±76.505	2.200±8.66E-00
4	85.667±3.055	799.333±12.342	2.133±0.057E-06
5	88.667±1.528	819.000±73.668	2.200±8.66E-08

Table 5: Mean±SD of Important Characters of Silkworm Hybrid F (NBxPM) x NB in 6 Generations

Generations	ERR (%)	FILL (ft.)	DEN
1	89.333±2.082	970.000±55.678	2.300±1. 84E-08
2	82.667±3.512	991.667±105.614	2.200±8.66E-08
3	87.667±3.055	912.333±77.009	2.267±0.057E-06
4	81.667±20.82	937.000±54.745	2.233±0.057E-06
5	86.667±3.055	974.333±109.792	2.300±1.84E-08
6	86.333±3.512	962.333±57.709	2.300±1.84E-08

Regression of ERR on generation number was not significant in any of the races. When the mean performance of KA (81.0±1.24) with F_5 (KA x PM) x KA (85.0±1.63) was compared. The magnitude is marginally higher in the hybrid (Tables 6 and 3). When the mean performance of NB_{18} (85.0±1.63) with F_5 (NB_{18} x PM) x NB_{18} (86.3±3.512) was compared, the magnitude was marginally higher in the hybrid (Tables 7 and 5).

Table 6: Mean ± SD of Important Characters in Bivoltine Silkworm Race KA over 12 Generations

Generations	ERR (%)	FILL (ft.)	DEN
1	82.000±2.160	873.333±30.912	2.200±0.081
2	82.667±1.247	868.333±37.043	2.167±0.047
3	72.667±2.054	897.667±19.258	2.233±0.047
4	72.333±0.1699	890.333±78.359	2.200±0.081
5	73.667±4.988	847.333±37.240	2.267±0.047
6	80.667±1. 699	875.000±29.337	2.233±0.047
7	74.000±3.741	849.333±45.616	849.333±45.616
8	83.000±2.44	901.667±6.236	2.167±0.047
9	69.667±3.681	969.333±35.349	2.233±0.094
10	948.667±79.264	2.367±0.047	2.367±0.047
11	78.333±1. 699	948.667±43.858	2.233±0.047
12	85.000±1.632	868.333±32.273	2.200±0.141

Table 7: Mean ± SD of Important Characters in Bivoltine Silkworm Race NB_{18} over 12 Generations

Generations	ERR (%)	FILL (ft.)	DEN
1	72.333±5.734	1023.667±29.780	2.567±0.047
2	75.333±7.318	997.667±9.809	2.200±0.141
3	72.000±2.943	898.667±55.535	2.167±0.047
4	76.000±2.943	1007.333±16.438	2.267±0.047
5	83.667±0.942	917.333±76.080	2.367±0.047
6	84.667±1.247	1013.000±10.230	2.367±0.047
7	74.667±3.681	944.333±66.890	2.367±0.047
8	83.000±2.449	923.667±46.334	2.267±0.047
9	69.667±3.681	969.333±35.349	2.233±0.094
10	81.333±1.247	948.667±79.264	2.367±0.047
11	78.333±1.699	948.667±43.858	2.233±0.047
12	85.000±1.632	868.333±32.273	2.200±0.141

Furthermore, it may be seen from table 8, the degree of heterosis calculated for the curves KAxPM over MP value (13.907) and BP (4.87) was significant. For the cross F_5 (KAxPM) x KA over MP (10.45) was found to be significant but it did not show any significance over better parent (Table 9). For the cross NB_{18}xPM the degree of heterosis was found to be significant over MP (14.625) and over BP (11.982) and for the cross F_5 (NB_{18}xPM) x PM did not show any significant heterosis.

Table 8: Heterotic Effects in Different Economic Characters of Silkworm Hybrids KAxPM

KAXPM	ERR (%)	FILL (ft.)	DEN
MP	13.907*	35.670*	2.246*
BP	4.878*	1.183	0.000

*Significance at 5%

Table 9: Heterotic Effects in Different Economic Characters of Silkworm Hybrids F_5 (KAXPM) X KA

F_5 (KAXPM) X KA	ERR (%)	FILL (ft.)	DEN
MP	10.45*	7.67	5.85*
BP	2.71	4.17	2.78

*Significance at 5%

Filament Length

The F_1 hybrids of the cross KAxPM revealed a mean filament length of 883±35.78 meters and it decreased in successive generations. In F_5 it was found to be 792+34.40 meters. The F_5 females were back crossed to KA males. In F_1 generation the mean filament length was found to be 882±35.40 meters with slight variations in successive generations (Tables 2 and 3). Later it was increased in F_5 and F_6 generations. From Fig. 3 the curve shows a decreasing trend in both two-way and three way crosses.

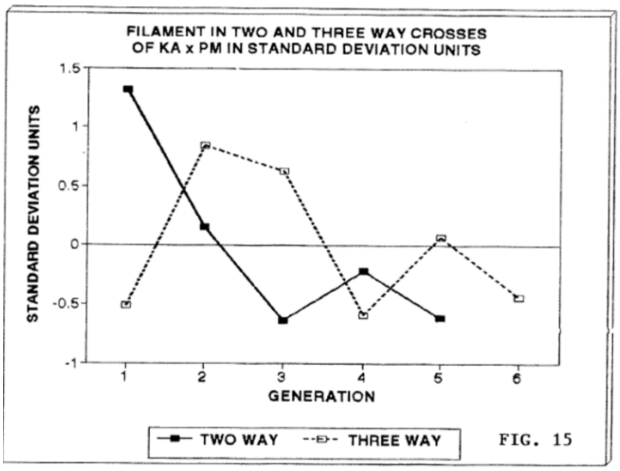

Figure 3

The F_1 hybrids of the cross NB_{18}xPM revealed a mean filament length of 878±41 meters which decreased in succeeding generations. The F_5 females were backcrossed to NB_{18} males. In F_1 the filament length was found to be 970±55 meters. It increased in F_2 generations, later decreased up to F_4 generations and in F_5 and F_6 it was found to be almost same showing the fixation of the trait leading to the isolation of the line R_2 (Tables 4 and 5).

From Fig. 4, it may be seen that the curve shows very less variations and increasing trend in both two-way and three way crosses which is expressed in terms of standard deviation units. Regression of filament length as generation's number is not significant in any of the races. When the mean performance of KA (868.6±36.917) with F_5 (KA x PM) x KA (886.0±36.615) was compared. The magnitude is marginally higher in the hybrid (Tables 6 and 3).

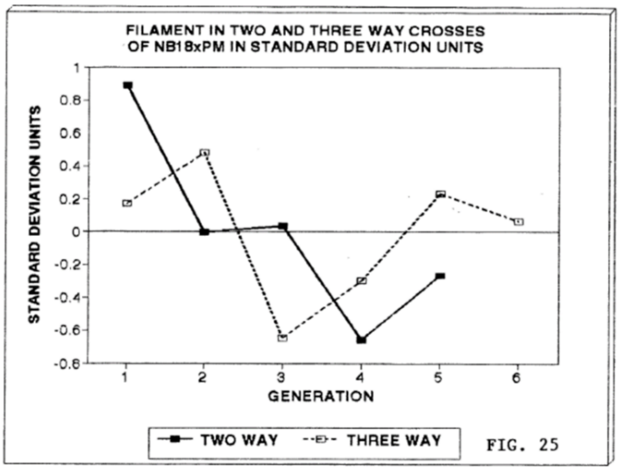

Figure 4

When the mean performance of NB_{18} (868.3±32.27) with F_5 (NB_{18} x PM) x NB_{18} (962.3±57.7) was compared, the magnitude was marginally higher in the hybrid (Tables 7 and 5). From table 8, it is seen that degree of heterosis of the hybrids KAxPM over MP (35,670) was significant but non-significant over B.P. For the hybrid F_5 (KAxPM) x KA the degree of heterosis did not show any significance over MP and BP value (Table 9).

From table 10, it may be seen that degree of heterosis of the hybrids NB_{18}xPM over MP (20.67) is significant and negative over BP (-14.360). In the hybrids F_5 (NB_{18}xPM) x NB_{18} the degree of heterosis is significant over both MP and BP value (Table 11).

Table 10: Heterotic Effects in Different Economic Characters of Silkworm Hybrids NB_{18} XPM

Bt>	ERR (%)	FILL (ft.)	DEN
MP	14.625*	20.67*	-7.801*
BP	11.982*	-14.360*	-15.584*

*Significance at 5%

Table 11: Heterotic Effects in Different Economic Characters of Silkworm Hybrids F_5 (NB_{18}XPM) X NB_{18}

F_5 (NB_{18}XPM) X NB_{18}	ERR (%)	FILL (ft.)	DEN
MP	3.66	11.73	0.656
BP	0.752	5.74*	2.95*

Denier

The F_1 hybrids of the cross KAxPM revealed a mean denier of 2.2 and it remained the same in succeeding generations and it decreased in F_5 to 2.1. The F_5 females were back crossed to the parent KA male with a mean denier of 2.2. In F_1 the denier increased to 2.3 and remained the same in all the generations showing the formation of the trait leading to the isolation of line R_1 (Tables 2 and 3).

From Fig. 5, it is seen that the curve shows declining trend in two-way cross and increasing trend in three-way cross. The F_1 hybrids of the cross NB_{18}xPM revealed a mean denier of 2.1 and it remained the same in F_2. Later it increased to 2.2 and remained the same in F_5. The F_5 females were back crossed to the parent NB_{18} males with a mean denier of 2.3 and in F_1 it was 2.3 and remained the same in F_2. In F_3 and F_4 it decreased to 2.2 and later in F_5 and F_6 it was 2.3.

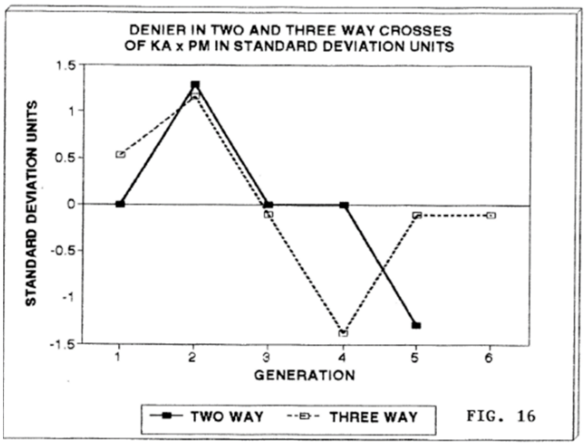

Figure 5

From Fig. 6, it is seen that the curve shows a zig-zag motion and an increasing trend in both two-way and three way crosses. Regression of denier on generation number was not significant in any of the races. When the mean performance of KA (2.2) with F_5 (KA x PM) x KA (2.3) was compared. The magnitude is marginally higher in the parent (Tables 6 and 3). When the mean performance of NB_{18} (2.2) with F_5 (NB18 x PM) x NB_{18} (2.3) was compared, the magnitude was marginally higher in the parent (Tables 7 and 5).

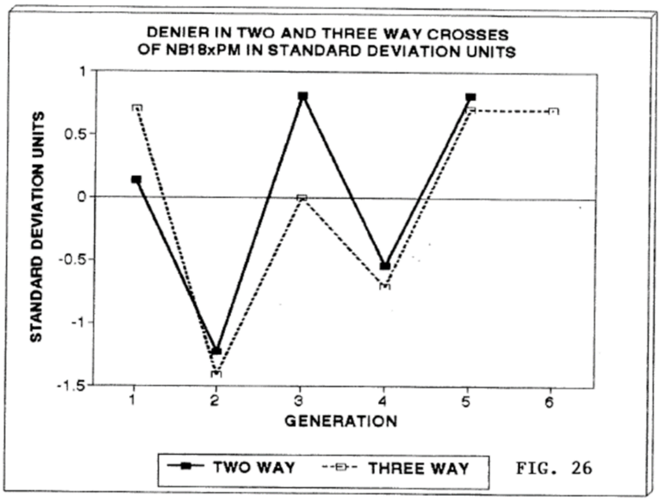

Figure 6

Furthermore, it may be seen from table 8, the degree of heterosis calculated over MP (2.246) was significant and it was nil over B.P for the cross KAxPM. For the cross F_5 (KAxPM) x KA, the degree of heterosis was significant over· MP (5.85) and it was not significant over B.P. (Table 9). It may be seen from table 10 the degree of heterosis calculated over MP (-7.801) was negative and significant and also over BP (-15.584) for the cross NB_{18}xPM. For the cross F_5 (NB18xPM) x NB_{18}, the degree of heterosis was significant over MP and BP (Table 11).

Crossbreeding is like a process that has been carried out since the beginning of civilization to improve any animal or plant breed for better performance than purebred breeds. It provides genetic manipulation options to select new and better combinations of genes. The use of known genotypes, selection of mating systems and careful selection methods are of decisive importance in conventional breeding methods, such as inbreeding and crossbreeding. Crossbreeding is more reliable than line crossing for the evolution of silkworm varieties. Some races are good for some characters and some are

not so good for certain characters. Mestizaje involves two or more races to obtain the desired character. Three hybrid lines with known genotypes were used to obtain promising new lines. The variety Mysore Yellow Tipped Multivoltine Spinning Pure exhibits disease resistance and is known for low silk yields.[11] Tropical Bivoltine Kalimpong A (KA) Spinning Oval white cocoon and NB18 Spinning Dumbbell White cocoons are known for their high yield silk more than students and relatively less resistant to diseases and adverse environmental conditions.[12]

In the present study, we used bivoltine females and multivoltine males. The crosses were made initially and backcrossed to F_5 generations. Analysis of the metric traits viz. ERR, filament length and denier revealed the manifestation of varied degrees of heterosis over mid parent and better parent. Owing to the simultaneous segregation of a large number of genes in conjugation with the influence of environmental effects. The results of the present study are in concurrence with various other researchers published in the literature. For instance, in a research study reported by Robertson (1966) revealed that Selection is made to choose intermediate sized cocoons from the batches of high ERR as these individuals are expected to possess maximum fitness value.[13] Accordingly inbreeding and selection over generations resulted in favourable combinations of desired alleles in the progenies leading to the expression of statistically non-significant differences for the metric traits under studies at later generation indicating their fixation in the isolated lines.

Furthermore, Kobayashi et al. (1968) who have reported an appearance of higher heterosis for productivity traits compared to viability traits.[14] Similarly, Mousseau and Roff (1987) have pointed out that the characters associated with viability are less heritable than the character associated with productivity which shows moderate to high heritability.[15] Petkov (1980) has reported the influence of the super dominant or incomplete genes on the expression of cocoon yield by weight, single cocoon weight, and pupation rate.[16] Therefore, the lower heterosis recorded for viability character and higher/moderate heterosis recorded for productivity characters can be attributed to lower/moderate/higher heritability for the respective characters.

Furthermore, perusal of heterosis on the importance and value of heterosis has undoubtedly established the fact that inbreeding, which results in increasing homozygosis provides a means for fixation of genetic characters, while out breeding emphasizes the practical value of heterozygosis, wherein the possibility superiority is established for fixation of genetic traits. It with is least obvious that heterosis in terms of specific phenotypic consequences therein would depend, on which loci are involved in the interaction of different alleles of the parents.

The results of the present investigation with regard to the manifestation of heterosis are in agreement with the findings of Osawa and Harada (1944).[17] Harada (1956, 1961) who have pointed that greater them id parent value, lesser will be the effect of heterosis. Overall performance of back cross hybrids are observed to be better than biparental hybrids (KAxPM or NB18xPM) due to the fact that backcross hybrids have 75% of the genetic structure resembles that of either KA or NB 18 as the case may be. While the biparental hybrid contains only50%ofNB18or KA.[18,19] However, degree of heterosis is more prominent in Biparental cross than backcross hybrids.[20]

CONCLUSION

Based on the results of metric traits *viz*. ERR, filament length and denier it was delineated that selection may be made to choose intermediate sized cocoons from the batches of high ERR because these individuals are expected to possess maximum fitness value.

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